HISTOLOGY AND HISTOCHEMISTRY OF THE CAPSULE OF *PROTEOCEPHALUS* SP. (CESTODA) IN *LEPOMIS MACROCHIRUS*[1]

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ABSTRACT

Histochemical studies performed on livers of the bluegill, *Lepomis macrochirus*, containing the larval cestode, *Proteocephalus* sp., indicated that the cellular capsule surrounding the parasite was composed of glycogen, basic protein with large concentrations of protein-bound amino groups, and tyrosine. An amorphous layer between the capsule and parasite gave positive reactions for mucoproteins, basic proteins, tyrosine, and tryptophan. The capsule was derived from reticulo-endothelial cells of the host, and the host plasma was the source of the amorphous layer.

INTRODUCTION

Although reports of the presence of the tapeworm, *Proteocephalus* sp., in livers of freshwater fishes have been discussed (Wardle and McLeod, 1952), investigations on host capsules have not been reported. The only histochemical studies on capsules produced by sunfishes were reported by Bogitsh (1961, 1962) on the acanthocephalan, *Neoechinorhynchus cylindratus*, and the trematode, *Posthodiplostomum minimum*. The purpose of this study was to determine the origin, histology, and histochemistry of the capsule surrounding the cestode, *Proteocephalus* sp., in livers of the bluegill, *Lepomis macrochirus*.

MATERIALS AND METHODS

Fifty northern bluegills, *Lepomis macrochirus*, were collected from ponds in Delaware County, Ohio. The livers were removed and fixed in 10% formalin, Zenker's fluid, or aqueous Bouin's solution for histological studies. Histochemical fixatives included Carnoy's fluid for carbohydrates and aqueous Bouin's solution for proteins. Some plerocercoids, dissected from infected livers, were fixed in formalin—alcohol-acetic acid for whole mounts. Infected livers were dehydrated in alcohol, cleared in benzene, and then infiltrated and embedded in paraffin at 56°C for 6 hours. They were sectioned at 5 to 8 microns. Unless otherwise stated, histological and histochemical procedures were as described by Humason (1962) and Lillie (1965).

Whole mounts were stained with Grenacher's borax carmine (Humason, 1962) and counterstained with fast green. Hansen's iron-hematoxylin (Lillie, 1965) and eosin, Mallory's triple (Humason, 1962), and Gomori's trichrome (Humason, 1965) stains were used to study the histology of the capsule. The presence of collagen was determined by Romeis' orcein (Lillie, 1965). Carbohydrate distribution was shown by the periodic acid Schiff reaction (PAS) of McManus (Lillie, 1965). Control sections were stained with Schiff's reagent without oxidation by periodic acid to test the presence, in the tissues, of those free aldehyde groups which interfere with the interpretation of the PAS test.

Two procedures were followed to demonstrate glycogen. One group of sections was digested in a 1.0% aqueous solution of malt diastase (Lillie and Greco, 1947) for 30 minutes prior to PAS staining. Another group was stained by Bauer's Feulgen method (Humason, 1962), using diastase as a control. Other sections were treated with a mixture of equal amounts of methanol, acetone, and chloroform

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at 60°C for 24 hours, and then stained by the PAS technique to determine the presence of lipid-carbohydrate complexes. Presence of mucoid substances was demonstrated by the following procedures: (1) Mayer's mucicarmine stain (Humason, 1962), for a general distribution of mucins; (2) PAS staining, preceeded by the Fisher-Lillie methylation technique (Lillie, 1965), for carboxyl groups in mucopolysaccharides; (3) Mowry's alcian blue modification (Humason, 1962); and (4) the metachromatic stain, toluidine blue, for acid mucopolysaccharides. The PAS reaction was used also to demonstrate the presence of glyco-proteins.

Test sections were digested with 0.1% pepsin in 0.01N HCl for 4 hours at 40°C (Gersh and Catchpole, 1949) and then stained by the PAS test. Controls were stained by the PAS reaction without digestion. Tissue elements giving negative reactions in test sections and positive reactions in the controls indicated glycoproteins. Proteins were detected by mercuric bromphenol blue (Mazia, Brewer, and Alfert, 1953) and basic proteins were demonstrated by omitting the mercuric salt from the reagent. Protein-bound amino groups were shown by the ninhydrin-Schiff reaction (Lillie, 1965). The Millon reaction (Humason, 1962) demonstrated tyrosine distribution and the Romieu reaction (Lillie, 1965) determined the presence of tryptophan.

RESULTS

All of the 50 livers of bluegills examined contained encysted juveniles of the acanthocephalan, *Neoechinorhynchus cylindratus*. Livers from 35 of these 50 fishes were infected also with plerocercoids of *Proteocephalus* sp. Each larval tapeworm was surrounded by a cellular capsule containing spindle-shaped cells with elongated nuclei. The wall of the capsule was three to five cells thick and measured 7.0 to 11.0 microns. Liver cells adjacent to the capsule appeared more vacuolated than the remainder of the parenchyma. Both the capsule and the liver, tested with Mallory's and Gomori's stains, indicated the presence of connective tissue, but collagen was not demonstrated.

These tissues also gave positive PAS reactions. The PAS and Bauer-Feulgen reactions did not occur following treatment with diastase, indicating the presence of glycogen in the tissues. Methanol-acetone-chloroform, which disolves all lipid components in tissues, did not alter the PAS reaction in the sections examined, ruling out the presence of lipid-carbohydrate complexes. Acid mucopolysaccharides were not present in either the capsule or host liver tissue, as shown by negative reactions with alcian blue and toluidine blue. Mucoproteins or glycoproteins were found in the hepatic portal and central veins of the liver, but these substances did not occur in the capsule or in the liver parenchyma.

Protein stains also showed a similarity between the liver and capsule. Both tissues were composed primarily of basic protein and contained large concentrations of protein-bound amino groups. Tyrosine was present and tryptophan was absent in both the capsule and the liver.

An amorphous layer occurred between the capsule and the cuticle of the tapeworm. This substance was eosinophilic, in contrast to the basophilic capsule. A positive mucoid reaction was given by Mallory's and Gomori's stains. Collagen was not demonstrated. Both the amorphous material and the cuticle of the parasite gave positive PAS reactions, although the color was not as intense as in the capsule and the liver. These results indicated that the carbohydrate component of the amorphous region differed from that of the capsule and liver parenchyma. The amorphous material did not contain acid mucopolysaccharides, although these substances were demonstrated in the cuticle of the parasite. Therefore, this material did not originate by a sloughing off of the cuticle, but probably came from the host material. This hypothesis is supported further by the presence of mucoproteins or glycoproteins in the amorphous material and an absence of these substances in the cuticle. The amorphous material gave a positive

FIGURE 1. Section through the liver (L) of *Lepomis macrochirus* showing the capsule (C) and amorphous material (A) surrounding the parasite *Proteocephalus* sp. (P). (×183).

reaction for basic proteins and negative reactions for protein-bound amino groups, tyrosine, and tryptophan.

DISCUSSION

The presence of fibroblasts, glycogen, protein-bound amino groups, and tyrosine in both the capsule and the liver indicate that cells associated with the liver are responsible for the formation of the capsule of *Proteocephalus* sp. This structure is similar in morphology and staining reactions to external cyst layers described by Bogitsh (1961, 1962) in *Neoechinorhynchus cylindratus* and *Posthodiplostomum minimum*. Results of this study are also in agreement with Bogitsh (1962), who reported that the outer cyst layer is derived from reticulo-endothelial cells of the host. Bloom and Fawcett (1962) state that monocytes, lymphocytes, and macrophages can give rise to fibroblasts in tissue cultures. Smyth (1966) states that vertebrate tissue reactions to parasites are similar to inflammatory reactions to foreign bodies, in which lymphocytes are transformed into fibroblasts. The transformation of blood cells into fibroblasts in insects and other invertebrates containing tissue parasites is reported by Salt (1963). Bowen (1967) found a similar reaction in millipedes infected with the acanthocephalan, *Macracanthorhynchus ingens*. In all instances involving parasites, the authors report fibrous capsules enveloping the organisms. The amorphous material between the capsule and parasite is a carbohydrate-protein complex in the form of a mucoprotein or glycoprotein. This substance was not demonstrated in the capsule, liver parenchyma, or cuticle of the parasite. The hepatic circulatory system of the host is the only other region on the sections showing glycoproteins, a fact indicating a relationship between the amorphous material and the host. I therefore feel that the host plasma, and not the reticulo-endothelial cells of the parasite, is the source of the amorphous material.

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